SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY NANDED

SYNOPSIS ON

"HPTLC Analysis and Bioefficacy of Some Selected Medicinal Plants of Rubiaceae from Kinwat Forest."

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HPTLC Analysis and Bioefficacy of Some Selected Medicinal Plants of Rubiaceae from Kinwat Forest.

Introduction:

Plants are the richest resource of drugs of traditional systems of medicine, modern medicine, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization (Jadon et. al. 2014). India is rich in medicinal plant biodiversity; all known types of agro-climatic, ecologic and edaphic conditions are met within India. Now, 80% of the world's population depends on traditional medicines for its primary health care needs. Plants remedies are often used as an alternative to allopathic medicines. Local people have discovered the therapeutic activity of medicinal plants against certain diseases through their indigenous experiences transferred to them from their ancestors. A large number of plants are being used in medicine for therapeutic or prophylactic purposes. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins (Jafra Bano et. al. 2015).

In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific aliments has been in vogue since ancient times. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. About 80 % individuals from developing countries rely on plant based preparations used in their traditional medicinal system and as the basic needs for human primary health care. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Medicinal plants have played important role in the traditional and orthodox system of medicine in the curing of different types of diseases.

Plants have proved to be significant natural resources for medicines; documentation of their use in medicine originates from ancient times. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries. Medicinal plant based drugs have the added advantage of being simple,

effective and offering a broad spectrum of activity. Herbal drugs are without any side effects besides being cheap and locally available. It has been reported that the antimicrobial activities of medicinal plants can be due to the presence of phytochemicals such as alkaloids, flavonoids and terpenoids. In recent years in order to discover novel antimicrobial drugs, screening of plants has been accelerated.

Phytochemicals are non-nutritive, chemical compounds that occur naturally on plants and have diverse protective properties (Minakshi B. 2016). Most Phytochemicals like carotenoids, flavonoids and polyphenols have antimicrobial activity and serve as a source of antimicrobial agents against human pathogens.

Study area includes the forest range of Kinwat taluka of Nanded district. Geographical position of the Kinwat is 19^0 25 to 19^0 55 N latitude and 77^0 51 to 78^0 19 E longitude. Forest cover in Kinwat is 57,800 hectares which is near about 39.34% of total forest cover of the taluka Kinwat has old heritage of medicinal plants and herbal medicine. Forest is rich in biodiversity and consists of rare medicinal plants. Biogeographical condition such as altitude, soil type and average rain fall make the vegetation rich in this area. The local tribes Andh, Gond, Kolam, Naikde and pradhan use forest resources for their day to day life (Kanthale and Biradar, 2012; Biradar, and Ghorband, 2012)

The family Rubiaceae is widespread and occurs in all major regions of the world. The family comprises 13,143 species falling under 611 genera by which it is the fourth largest Angiosperm family (Davis et al. 2009). The Rubiaceae family is characterized by the production of bioactive metabolites with great pharmacological potential. The Rubiaceae family is characterized by the production of bioactive metabolites with great pharmacological potential. In recent years Rubiaceae species have been thoroughly studied from a phytochemical view point Daiane Martin *et. al.* (2015). According to Naik V. N. (1998) in Marathwada the Rubiaceae is represented by 18 genera and 38 species

Review of literature:

Nature has a source for several medicinal components that favour human for thousands of years and an impressive number of modern drugs have been isolated from such a natural sources; most of these modern drugs are isolated from the traditionally used ones. This plant-based medicine system is playing an essential role in the healthcare.

Historically, the pharmacological screening of compounds either from natural or synthetic origin are the sources of innumerable therapeutic agents. Random screening is a method for discovering new biologically active molecules and is more productive in the field of antibiotics Medicinal plants represent a rich source of antimicrobial agents. Plants are used as medicines in different countries and are a source of many potent and powerful drugs. Although hundreds of plant species have been tested for biochemical properties, majority of the plants are yet to be examined for biological and chemical properties Poornimaet.et. al. (2014). Thus by considering the same view we have planned to study some plants of Rubiaceae from our region.

Karou et. al. (2011) studied on traditional uses, phytochemistry and biological activities of medicinal plants from Rubiaceae family. Also they have studied that more than 60 species of Rubiaceae family which are used for more than 70 medicinal indications including malaria, hepatitis, eczema, oedema, cough, hypertension, diabetes and sexual weakness.

Raghavendra *et. al*. (2017) investigated antimicrobial, antiradical and insecticidal potential of leaf and fruit of *Gardenia gummifera* L. (Rubiaceae). They have reported that the fruit extract of *G. gummifera* exhibited marked antimicrobial, antiradical and insecticidal activity when compared to leaf extract. The plant can be used for developing agents/formulations effective against infectious microorganisms, oxidative stress and insect vectors that transmit dreadful diseases.

The study made by Damle and Sharon (2017) in Karwar, Maharashtra on the phytochemical constituents and bioactivity of *Ixora coccinea* Linn. showed the presence of class of compounds which is known to possess anti- microbial activity. TLC fingerprint reveals a number of compounds, as observed under 254nm and post derivatization with anisalydehyde at 366 nm. Bioautography studies suggests that the plant has potential bioactive compounds. Accordingly the TLC fingerprint can be used to authenticate herbal samples containing *Ixora coccinea* Linn.

Hemant and Mahendra (2017) from Udaipur made a study on preliminary phytochemical analysis and antioxidant activity of ethanolic extract of *Woodfordia fructicosa* flower sand *Gardenia gummifera* leaves. The DPPH radical scavenging activity of both the extracts increases with increasing concentration of extract. The

investigation done supports the use of *Woodfordia fructicosa* and *Gardenia gummifera* in traditional treatment of number of diseases.

Objectives of research:

The present research work is aimed with following objectives.

1. Ethnobotanical survey and to record the indigenous uses of medicinally important plant resources of Rubiaceae.
2. Phytochemical analysis of some selected plants of Rubiaceae.
3. Determination of antimicrobial potential of selected plant extracts.
4. Determination of antioxidant/free radical scaving activity of some selected plants
5. Determination of antidiabetic and anticancerous activity of some selected plants
6. HPTLC analysis of collected plants.

Methodology to be adopted:

The current research work will be completed using standard and established protocols.

Phase I: Literature Survey, collection and identification of Plant Materials.

In the first phase of research work extensive literature survey will be done in order to know latest scenario of research in undertaken and allied area. The ethno botanical survey and collection of plant material will be done by giving personal visit to field and also by interacting with some local tribal healers. The collected plant materials will be identified by using standard floras (Cooke 1967; Naik 1998).

Phase II:Plant Extraction and phytochemical analysis.

Plant samples will be collected from Kinwat forest in different seasons. The samples will be extracted for phytochemical analysis by standard and prescribed methods. (Harborne,1973;Sadasivam & Manikam, 2005; Thimmaiah, 1999, Evans 1997).

Phase III:Antimicrobial Studies.

By using standard protocols in third phase antimicrobial activity of plant extracts of Rubiaceae will be tested against different fungi and bacteria.

Phase IV:Antioxidant, Antidibetic and Anticancerous activity.

In this phase the focus will be given on Antioxidant, Antidibetic and Anticancerous activity using standard protocols recommended by, Gulnaz et. al. (2014); Abdullah and Kashim, (2017); Anticancerous activity will be carried out employing

outsourcing facility available at specific scientific research labs (Rubinstein and Boy, 1990).

Phase V: HPTLC Analysis

In this phase emphasis will be given on HPTLC analysis of selected plant extracts in central instrumentation facility available in Science College Nanded using standard protocols (Sethi 1996; Mona *et. al.* 2012).

Phase-VI: Thesis writing and Submission.

Scientific writing of thesis will be done for final submission to University.

Importance of study / society application:

International status:

At International level efforts are being made to evaluate biological and chemical potential of one of the largest family of angiosperm, the Rubiceae. In a nutshell we are citing some references here in this synopsis.

Mohamed Zaki Zayed et. al. (2014) made GC-MS analysis of phytochemical constituents in leaf extracts of *Neolamarckia cadamba* from Malaysia. They reported that the plant is of phytopharmaceutically important. Its leaves can be used as antimicrobial, antiparasitic insecticide, nematicide, pesticide, antiandrogenic, hypo cholesterolemic, antioxidant, cancer preventive, anticoronary, antiarthritic, hepatoprotective, neuroactive, analgestic and anesthetic, flavour and allergenic. The presence of various bioactive compounds has justified the use of *N. cadamba* leaf extracts for various ailments by traditional practitioners.

Tiwtawat et. al. (2017) from Colombia studied antibacterial activity of three medicinal Lasianthus (Rubiaceae) extracts on human resistant pathogenic bacteria. The work which supports the traditional uses related to treating infectious disease and showed antibacterial effects against bacterial pathogens which are serious health problems. Qualitative analyses of lipophilic leaf and stem bark extracts from the three Lasianthus were also studied by usingchromatographic techniques (TLC and HPLC).

Gustavo et. al. (2017) in Brazil has made phytochemical study on *Psychotria stenocalyx,* a plant of Rubiaceae. They have reported seven monoterpeneindole alkaloids were identified: lyaloside1, (E)-O-(6′)-cinnamoyl-4”-hydroxy-3”,5”-dimethoxy-lyaloside 2, strictosamide3, pauridianthoside4, vallesiachotamine lactone 5, E-vallesiachotamine6

and Z-vallesiachotamine7, in the alkaloid fraction, obtained by an acid-base extraction from crude extract.

National status:

In relation to the national status related to the bioefficacy of Rubiaceae, several Indian researchers have made immense contribution in this field from long time, few of them are mentioned in this synopsis.

Poornima et. al. (2014) from Chennai studied antibacterial activity of the Leaf Extracts of different varieties of *Ixora coccinea.* They found that ethyl acetate extracts of all varieties showed significant concentration-dependent antibacterial activity, particularly, against gram negative bacteria. Bioassays showed the presence of several specifically active compounds at different Rf values in *Ixora coccinea* red plant leaf extract. Presence of phytochemicals such as alkaloids, phenols, steroids, saponins, and flavonoids were observed in the plant extracts which have antibacterial activity and can be used for medicinal purpose.

Sirigiri Chandrakala (2015) from Andhra Pradesh, India studied medicinal attributes of family Rubiaceae. They have studied the importance of phytochemical, antimicrobial and pharmacological activities of Rubiaceae species.

Pratibha et. al. (2016) from Uuttrakhand studied polyphenolic content and antioxidant activity of *Adina cordifolia* of Rubiaceae. The methanolic extract of leaf, bark and root were first time subjected to different antioxidant assays such as radical scavenging assay, total antioxidant assay and estimation of polyphenolic contents. Methanolic extract of leaf showed the highest total antioxidant activity i.e., 1.43±0.087. The IC50 values of methanolic extract of leaf for DPPH radical scavenging activity and nitric oxide scavenging activity was found to 48.4 and 110.5 µg/ml respectively. Polyphenolic contents were also found higher in methanolic extract of leaf. The active antioxidant compounds were found higher in methanolic extract of leaf which showed a direct correlation between the total polyphenols extracted and its anti-oxidant activity.

Samudra S. (2016**)** has studied ethnomedicinally important plants of family Rubiaceae from Pune district, Maharashtra. They have studied 8 different plants from Rubiaceae family which are used for medicinal purpose. Majority of plants of family

Rubiaceae are used to cure diarrhea, dysentery, fever, wounds, bronchitis, dysepepsia, joint diseases etc.

Gulnaz et. al. (2014) from Karnataka has done Phytochemical screening of Ethanolic extract of *Randia uliginosa.* The result showed a wide variety of Phytoconstituents such as alkaloids, saponins phenols, flavonoids, tannins etc, antimicrobial activity evaluated against four clinical isolates *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillum niger* maximum activity was observed against *Staphylococcus aureus*(MIC0.187mg/ml) followed by *Pseudomonas aeruginosa* and the fugal isolates (MIC 0.375mg/ml), and antioxidant potential showed its ability to scavenge various free radicals like DPPH, Super oxide and the Hydroxylradicals. The results obtained provide a scientific evidence for the traditional use of *Randia uliginosa* for the treatment of various diseases.

Naira Nayeem et. al. (2011) from Banglore, Karnataka evaluated methanolic extracts of the leaves of *Coffea arabica* (CA) and *Coffea robusta* (CR) for its antioxidant and antimicrobial activity. The total phenolic content, flavonoid content and condensed tannin content were determined by the Folin ciocalteu method, Aluminum chloride colorimetric method and vanillin assay respectively. Antimicrobial activity was determined by using cup diffusion assay while the antioxidant activity was evaluated using DPPH. The total phenolic content was found to be 27.04 μg/g and 21.80 μg/g, total flavonoid 10.90 μg/g and 8.08 μg/g, and condensed tannins 3.1 and 2.9 respectively for *Coffea arabica* (CA) and *Coffea robusta*(CR) respectively. *Coffea robusta* showed better antimicrobial and antibacterial activity when compared to *Coffea arabica*.

Significance of the study:

Plants have been used for medicinal purposes long before prehistoric period. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes. Human beings are depending on botanicals directly or indirectly for the treatment of various ailments since ancient time, according to the World Health Organization, reports nearly 80% psychological and physical health mainly because of the two reasons one is they cannot afford the products of Western pharmaceutical

industries and the second reason is due toside effects of the synthetic drugs followed by lack of healthcare facilities. India has a rich culture of medicinal herbs and spices, with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been investigated chemically and pharmacologically for their potential medicinal value. Rural masses of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life. These medicines are relatively safer and cheaper than synthetic or modern medicine. Gulnaz et. al. (2014). In current study proposed ethnobotanical survey will help in knowing traditional knowledge of Rubiaceous flora of human welfare. The scientific and biological studies planned will be useful in knowing active principles present in the plants. Chromatographic characterization of Rubiaceous flora from Kinwat forest will be applicable in identification and authentication of plant drugs. Antioxidant, anticancerous and antidiabetic study proposed will find its application in herbal treatments of these diseases.

Proposed work Plan/ Formulation and Structure of Study: Year-wise Plan of work and targets to be achieved

The current research work planned for three years as follows.

First year:

1. Literature Survey/Review of Literature and geographical study of Kinwat forest.
2. Ethnobotanical survey, collection and identification of Plants
3. Extraction of plant materials by using different solvents.

Second year:

1. Phytochemical analysis of selected plants.
2. Antimicrobial activity of selected plant extract.
3. Antioxidant and antidiabetic activity of selected plants.

Third year:

1. Anticancerous activity of selected plants.
2. HPTLC analysis of plant extracts.
3. Data analysis and thesis writing for submission to the University.

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